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Biomarkers, Metabonomics, and Drug Development: Can Inborn Errors of Metabolism Help in Understanding Drug Toxicity?

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Subrahmanyam Vangala[1] and Alfred Tonelli[1]

[1]Global Preclinical Development, Johnson & Johnson Pharmaceutical Research and Development, Raritan, NJ

ABSTRACT

Application of "omics" technology during drug discovery and development is rapidly evolving. This review evaluates the current status and future role of "metabonomics" as a tool in the drug development process to reduce the safety-related attrition rates and bridge the gaps between preclinical and clinical, and clinical and market. Particularly, the review looks at the knowledge gap between the pharmaceutical industry and pediatric hospitals, where metabonomics has been successfully applied to screen and treat newborn babies with inborn errors of metabolism. An attempt has been made to relate the clinical pathology associated with inborn errors of metabolism with those of drug-induced pathology. It is proposed that extending the metabonomic biomarkers used in pediatric hospitals, as "advanced clinical chemistry" for preclinical and clinical drug development, is immediately warranted for better safety assessment of drug candidates. The latest advances in mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy should help replace the traditional approaches of laboratory clinical chemistry and move the safety evaluation of drug candidates into the new millennium.

KEYWORDS: Biomarkers, clinical chemistry, drug development, inborn errors of metabolism, metabonomics, toxicity

INTRODUCTION

With increased safety standards from worldwide regulatory agencies, there is an increased need for better safety biomarkers. Traditional clinical pathology and hematology biomarkers have a proven record as indicators of clinical toxicity. However, the sensitivity of these is quite varied and is sometimes considered suboptimal, with changes generally occurring only after significant tissue damage has occurred. Additionally, most current biomarkers point to the target organ without revealing the mechanism of toxicity. The primary intention of this review is to present some of the well-known markers and clinical signs of inborn errors of metabolism and to suggest that they could be more broadly applied to recognizing and understanding the mechanisms of toxicities observed during drug development. Currently there are no examples in the literature that prove that drug-mediated pathologies are similar to the pathologies of inborn errors of metabolism. However, we feel that as researchers use their expanding knowledge about genomics and increasingly sophisticated analytical tools to understand and measure drug-mediated and endogenously mediated toxicities, valuable links will be found. The intent of this review is to expand this emerging research area and move us a step closer to understanding the complex mechanisms of drug-mediated toxicity.

A drug that is approved for human use must be both safe and efficacious. In fact, attrition of drug candidates in development is primarily due to safety concerns (30% combined preclinical and clinical) and lack of efficacy (30%).[1,2] As safety and efficacy standards are raised, the drug development process has become even more challenging. Once a drug reaches the market and moves into a larger and broader patient population, concerns about safety move to another level. Adverse drug reactions (ADR) occur in ~5% of all treated patients.[3] It has been estimated that between 2% and 20% of all hospital admissions are due to ADR, and ~10% of all hospitalized patients experience ADR during their hospital stay.[3] One in 5000 to 10 000 patients also die because of rare side effects, which are known as idiosyncratic drug toxicity (IDT).[4-6] Since the 1960s, more than 20 drugs have been withdrawn from the market because of either ADR or IDT.[7] Although physicians are obliged to report ADR, unfortunately, a substantial proportion of ADR are not reported because the association between the drug and the ADR is in doubt[3] or for other reasons.[8,9] In some cases, histopathological examinations are needed to determine the diagnosis of ADR.[3]

The mechanisms of ADR are poorly understood, but ADR can be divided into 2 main groups: immune-related and non-immune-related.[10] It appears that a significant portion of non-immune-related ADR is due to drug-induced metabolic disorders.[11-15] For example, the long-term toxicities of highly

Corresponding Author: Subrahmanyam Vangala, Toxicology and Pharmacology, SHIRE, Human Genetic Therapies, 700 Main Street, Cambridge, MA 02139. Tel: (617) 230-2746; E-mail: svangala@shire.com

active antiretroviral therapy include the syndrome of maldistribution, hyperlipemia, glucose tolerance, and insulin resistance.[11] Metabolic disorders represent 10.2% of ADR related to cardiovascular drug therapy in Germany.[12] Similarly, metabolic disorders represented ~12% of ADR in cardiologic hospitals in France.[13] Many of these "drug-induced" metabolic disorders have similarities with inborn errors of metabolism, but no clear evidence is available to indicate that the underlying mechanisms are similar.

Some of the drugs that cause ADR or IDT are bioactivated to reactive metabolites that covalently bind to proteins.[4-6,16-18] Some pharmaceutical companies' chemistry programs focus on the identification of reactive metabolites early in drug discovery to minimize the potential for bioactivation of compounds nominated to drug development. Although the reactive metabolites can be identified easily, the identification of proteins that are specifically modified relating to drug-induced specific toxicities has been futile. Furthermore, covalent binding to proteins by reactive intermediates does not necessarily correlate with ADR or IDT, suggesting that biological processing of the covalently bound proteins is rather complex. Alternatively, other unidentified mechanisms of toxicity may be involved.[17-19]

Some pharmaceutical companies are also investing in "omics" technologies (eg, genomics, proteomics, and metabonomics) in an effort to characterize the specific biomarkers that relate to either safety or efficacy. However, this research is still in its infancy. Future investments in these technologies may help (1) speed up the drug development process, (2) close the gaps between preclinical and clinical development, and (3) close the gaps between clinical development and market.

Despite the hope for biomarkers and the progress made so far, much challenge lies ahead. Consider that the total number of biomarkers of interest can be estimated to be ~1 133 000, of which genome accounts for ~25 000 to 30 000,[20] transcriptome 100 000,[21] proteome 1 000 000,[22] and metabonome ~2500 to 3000.[21,23] The fingerprinting approach to evaluating all these biomarkers and developing a rational hypothesis for a causal or casual association of biomarkers with efficacy or safety is currently proving to be very difficult. Although we may understand specific tasks or implications of biomarkers, biology and evolution have provided us with redundancies, cascading systems, and cross-talks among signal transduction events with overlaps that are often misleading.[24-26] Another complicating factor is the influence of the environment on transcriptional, translational, and posttranslational events, resulting in discordances in genotype and phenotype.[27] In addition, over time there have evolved generalized mechanisms of cell death,[28] including apoptosis and necrosis irrespective of cell type, tissue, or organ. Thus, the toxicogenomics approach of fingerprinting genes and identifying mechanism-specific toxicities has proven to be more difficult than anticipated. More effective interpretations can sometimes come from an integrated analysis of classic metabolic errors and markers.

Metabonomics and Intermediary Metabolism

Metabonomics is a powerful tool for identifying any disturbances in normal homeostasis of metabolic processes, including those involving carbohydrates, fatty acids, amino acids, and nucleic acids. These normal metabolic processes are highly regulated and are vital for cell structure and function. These functions are catalyzed by various enzymes, which are in turn regulated by specific genes. Defects in genes/enzymes could lead to chronic diseases in humans and in some cases mortality. How new is metabonomics? Sir Archibald Garrod pioneered the research on metabolic disorders in the early 1900s, discovering various diseases, including alkaptonuria, pentosuria, cystinuria, and albinism. To date, more than 150 inborn genetic disorders have been characterized; some of the more familiar inherited metabolic disorders (IMDs) are cystic fibrosis, hypothyroidism, sickle cell anemia, phenylketonuria, and Tay-Sachs disease. Garrod published *Inborn Errors of Metabolism,* the first book on the subject, in 1908.[29] He also formulated the 1 gene–1 enzyme hypothesis. As we progress further in understanding the human genome's functioning, many other inborn errors of inherited disorders may be discovered. For example, there have been recent discoveries that characterize defects in pyrimidine metabolism, cytochrome P450 enzymes, and hepatocanalicular transport proteins.

Metabolic disorders are rare events affecting about 1 in every 5000 babies born (note the similarity to the incidence of IDT, discussed above).[30] Many of the metabolic disorders have been characterized with well-defined clinical pathology, and specific "metabonomic" biomarkers have been identified.[31] Indeed, several pediatric hospitals screen newborn babies using mass spectrometry techniques with the known appropriate metabonomic biomarkers.[32-37]

Metabonomics offers several advantages over genomics and proteomics:

1. It has a relatively small number of biomarkers (~2500-3000).
2. It can be applied noninvasively in biofluids (plasma, urine, feces, etc), which can be considered "expanded clinical chemistry."
3. Combined toxicogenomic and metabonomic data in animal models can help focus on evaluating metabonomic biomarkers of clinical relevance.
4. Several diseases, particularly those from inborn errors of metabolism, have been characterized with

specific clinical biomarkers in relation to clinical pathology.

In this article, the literature on metabolic disorders is reviewed with particular emphasis on their relationship to known clinical pathologies and specific biomarkers characteristic of these diseases. An attempt is made to compare IMDs to drug-induced toxicity in preclinical animal models and idiosyncratic toxicity in humans. Conceptually, a drug or its reactive metabolites can inhibit or inactivate key enzymes that regulate vital metabolic processes, thus mimicking metabolic disorders and leading to drug-induced toxicities. Using a metabonomics approach as "advanced clinical chemistry" for preclinical safety studies and clinical trials may provide more sensitive biomarkers of drug toxicity. Although metabonomics may also be applied for understanding efficacy, this review focuses on only safety applications of metabonomics.

Inborn Errors of Metabolism/Inherited Metabolic Disorders

The well-characterized IMDs in humans[30] involve defects in enzymes catalyzing carbohydrate (Tables 1 and 2), amino acid (Table 3), fatty acid (Tables 4 and 5), nucleic acid (Tables 6 and 7), and urea cycle (Table 8) metabolism. Additional inherited disorders are linked to defects in cytochrome P450 enzymes (Table 9) catalyzing cholesterol,[38] bile acid,[39] steroid,[40-43] and vitamin D3[44] synthesis and metabolism. In recent years, genetic defects in transporters (Table 10) that mediate transport of endogenous metabolites and drugs have also been described with well-defined clinical pathology.[45,46] Given the significance of these enzymes and toxicological outcomes of their genetic deficiencies, a brief link to human genetic defects and their link to diseases will be provided. Tables 1 through 10 list IMDs along with their clinical pathology and also, where available, the biomarkers used for diagnosis of these diseases.

Clinical Manifestations and Laboratory Markers of IMDs

The clinical and laboratory manifestations of IMDs can include acute metabolic encephalopathy, hyperammonemia, metabolic acidosis, hypoglycemia, jaundice and liver dysfunction, lipid and liposomal storage disorders, and altered morphological features.

Acute Metabolic Encephalopathy

IMDs, most notably the organic acedemias/acidurias (Tables 4 and 5), urea cycle defects (Table 8), and certain amino acid metabolism disorders (Table 3), present with acute life-threatening symptoms of encephalopathy and measurable clinical pathology parameters.[31] These symptoms are primarily the result of the toxic effects of accumulating metabolites on the central nervous system (CNS). Other signs of CNS dysfunction, such as seizures and abnormal muscle tone, also occur. Cerebral edema and intracranial hemorrhage may occur occasionally. If untreated, these patients may progress to coma.

An infant with an IMD may present with first-onset symptoms such as apnea or respiratory distress. Apnea is typically central in origin and a symptom of the metabolic encephalopathy. Tachypnea may be a symptom of an underlying metabolic acidosis, as occurs in the organic acidemias. Infants with urea cycle defects (Table 8) and evolving hyperammonemic coma initially exhibit central hyperventilation, which leads to respiratory alkalosis. Vomiting is a striking feature of many of the IMDs associated with protein intolerance.

Hyperammonemia

Hyperammonemia is one of the most important findings in the neonate patient with IMD who has been diagnosed with

Table 1. Carbohydrate Metabolism Disorders

Deficiency	Disorder	Clinical Feature
Galactose-1-phosphate uridyl transferase	Galactosemia	Swollen and inflamed liver; jaundice; weight loss; hypoglycemia; kidney failure; ovarian failure in girls; stunted physical and mental growth; cataracts in eyes
Fructose-1-phosphate-aldolase	Fructosemia	Accumulation of fructose in liver, kidney, and intestine; dislike for sweets and fruits. Fructose intake results in abdominal pain, vomiting, and hypoglycemia. If untreated, can cause liver and kidney damage.
Hepatic fructokinase	Fructosuria	Increased fructose in blood and urine. Rare and harmless.
L-xylulose	Pentosuria	Increased L-xylulose in urine. Harmless. Occurs almost exclusively in Ashkenazim Jews of Polish-Russian origin.

Table 2. Carbohydrate Metabolism (Glycogen Storage) Disorders*

Deficiency	Disorder	Clinical Feature
Glucose-6-phosphatase (liver and kidney)	Von Gierke's disease (GSD type I)	Increased glycogen; massive enlargement of liver; severe hypoglycemia, ketosis, hyperuricemia; hyperlipemia; failure to thrive
α-1,4-Glucosidase (acid maltase)	Pompe's disease (GSD type II)	Increased glycogen; hypotonia; congestive heart failure, cardiomegaly; weak bulky muscles
Amylo 1,6-glucosidase (debranching enzyme; muscle and liver)	Cori's disease (GSD type III)	Increased glycogen with shorter outer branches; enlargement of liver; hypoglycemia; ketosis; hyperuricemia; hyperlipemia
Brancher enzyme (α1,4 α1,6) (liver and spleen)	Anderson disease (GSD type IV)	Normal levels of glycogen with very long outer branches; progressive cirrhosis of the liver. Hepatic failure causes death usually before age 2.
Muscle glycogen phosphorylase	McArdle disease (GSD type V)	Moderately increased glycogen; limited ability to perform strenuous exercise due to muscle cramps
Liver phosphorylase	Herr disease (GSD type VI)	Like type I, but milder
Muscle phosphofructokinase	GSD type VII	Similar to type V
Liver phosphorylase kinase	GSD type VIII	Increased glycogen; mild liver enlargement and hypoglycemia

*GSD indicates glycogen storage disorders.

acute encephalopathy. A plasma ammonia level is obtained for any child with unexplained vomiting, lethargy, or other evidence of an encephalopathy. In neonates, IMDs involving urea cycle defects (Table 8) and many of the organic acidemias (Table 4) are the primary cause of hyperammonemia, whereas in the older infant, fatty acid oxidation defects (Table 5) may be considered. Ammonia levels in newborns with these conditions frequently exceed 1000 μmol/L. The finding of marked hyperammonemia provides an important clue to diagnosis and indicates the need for urgent treatment to reduce the ammonia level. The degree of neurologic impairment and developmental delay observed subsequently in affected infants has been shown to be dependent on the duration of the neonatal hyperammonemic coma.

Hyperammonemia, along with respiratory distress and metabolic acidosis, is common in patients with some of the organic acidemias, such as glutaricacidemia type II (Table 4), pyruvate carboxylase deficiency, or urea cycle disorders (Table 8). Urine organic acid analysis is always obtained, regardless of whether acidosis is present. Plasma amino acid analysis is also obtained in the differentiation of specific defects in this group. In addition, carbamoyl phosphate synthetase deficiency and ornithine transcarbamoylase deficiency may be differentiated by measuring urinary orotic acid, which is low in the former and elevated in the latter (Table 8).

Metabolic Acidosis

The second most important laboratory feature of many of the IMDs during acute episodes of illness is metabolic acidosis with an increased anion gap (≥16), demonstrable by measurement of arterial blood gases or serum electrolytes and bicarbonate. Among the inherited disorders, the largest group typically associated with overwhelming metabolic acidosis in infancy is the group of organic acidemias, including methylmalonic aciduria, propionic acidemia, glutaric acidemia type II, and isovaleric acidemia (Tables 4 and 5). Plasma lactate is often elevated in organic acidemias as a result of secondary interference with coenzyme A (CoA) metabolism. Neutropenia and thrombocytopenia may be observed. Hyperammonemia, sometimes as dramatic as that associated with urea cycle defects, is commonly but not uniformly seen in clinically ill infants with organic acidemias.

On the other hand, defects in pyruvate metabolism or in the respiratory chain may lead to lactic acidosis, which presents in infancy as severe metabolic acidosis but with normal urine organic acids. Unlike most of the other conditions presenting acutely in the newborn, the clinical features of these disorders are unrelated to protein intake. Measuring the plasma lactate-to-pyruvate ratio facilitates differentiation of the various disorders in this group. A normal ratio (≤25)

Table 3. Amino Acid Metabolism Disorders

Deficiency	Disorder	Clinical Feature
Phenylalanine hydroxylase	Phenylketonuria	Accumulation of phenylalanine and its metabolites; low brain weight; defective myelination of nerves; death
Fumarylacetoacetate hydrolase	Tyrosinemia 1	Succinylacetone test in urine; increased α-fetoprotein in serum; poor weight gain; enlarged liver and spleen; distended abdomen; swelling of legs; tendency to have nosebleeds; diarrhea; cirrhosis; fatal hepatic failure
Tyrosine aminotransferase	Tyrosinemia 2	Tyrosinuria and tyrosyluria; elevated tyrosine metabolites; eye problems such as lacrimation, photophobia, redness, dendritic ulcers, neovascularization, cataracts, glaucoma, visual impairment; skin lesions—blisters or erosions leading to crust and hyperkeratosis, hyperhidrosis, painful palms and soles
Arginosuccinate lyase	Urea cycle disorder	Arginosuccinic acid in urine
Branched chain α-ketoacid dehydrogenase	Maple syrup urine disease	Leucine, valine, and alloleucine in plasma
Cystathionine synthase	Homocystinuria	Homocysteine in plasma, urine; eye disorders including ectopia lentis, staphyloma, cataracts, glaucoma, retinal detachment, and optic atrophy; musculoskeletal disorders; central nervous system disorders; progressive mental retardation; failure to thrive; seizures; psychiatric disorders; vascular disorders—thromboembolism
Homogentisic oxidase	Alkaptonuria	Homogentisic acid in urine (black urine); arthritis; brown-colored bone and cartilage

with increased pyruvate concentrations is indicative of pyruvate dehydrogenase (PDH) deficiency or defects in gluconeogenesis. Alternatively, a high lactate-to-pyruvate ratio (usually >35) with decreased or normal pyruvate concentrations is associated with total pyruvate carboxylase deficiency or respiratory chain defects. A high lactate-to-pyruvate ratio (usually >35) and decreased or normal pyruvate occurs with total pyruvate carboxylase deficiency (the usual form that presents in neonates) or respiratory chain defects and is associated with mitochondrial myopathy.

Hypoglycemia

Hypoglycemia and its associated symptoms are commonly seen in disorders of carbohydrate metabolism or fatty acid oxidation, although protein intolerance can also cause hypoglycemia. Among the best-known IMDs associated with hypoglycemia are the hepatic glycogen storage disorders (GSDs) (Table 2). The hypoglycemia in these disorders is related to the inability of the liver to release glucose from glycogen, and it is most profound during periods of fasting. Hypoglycemia, hepatomegaly, and lactic acidosis are prominent features of these disorders. Hypoglycemia is not a feature of GSD type II (Pompe's disease) because cytoplasmic glycogen metabolism and release are normal in this disorder in which glycogen accumulates within lysosomes as a result of deficiency of the enzyme acid maltase (α1, 4-glucosidase). Clinical manifestations of this disorder include macroglossia, hypotonia, cardiomegaly with congestive heart failure, and hepatomegaly with cardiomegaly as the most striking feature in the neonate (Table 2). Congestive heart failure is the cause of death in most cases before age 2. Hypoglycemia may also be a prominent feature of both galactosemia and hereditary fructose intolerance (Table 1), although symptoms of the latter disorder occur only after fructose (sucrose) is present in the diet.

Table 4. Organic Acedemias/Acidurias*

Deficiency	Disorder	Clinical Feature
Propionyl-CoA carboxylase	3-Hydroxypropionic acid, tiglylglycine; methylcitrate in urine; ammonia in plasma; hyperglycinemia and hyperglycinuria	Metabolic acidosis; vomiting; lethargy; ketosis; neutropenia; thrombocytopenia; hypogammaglobulinemia; developmental retardation; intolerance to protein
Isovaleric-CoA dehydrogenase	Isovaleric aciduria—C5; 3-hydroxyisolvaleric acid; isovalerylglycine in urine	Metabolic acidosis; severe ketoacidosis in adults
3-Methylcrotonyl-CoA carboxylase	3-Methylcrotonylglycine; 3-hydroxyisolvaleric acid; 3-hydroxyisovalerylcarnitine in urine	Metabolic acidosis; hypotonia, muscle atrophy; seizures and dermatological changes
Methylmalonic-CoA mutase	Methylmalonic aciduria; ammonia and homocysteine in plasma	Metabolic acidosis; lethargy; failure to thrive; vomiting; dehydration; respiratory distress; hypotonia; hepatomegaly; seizures; stroke; developmental delays; coma
Multiple Acyl-CoA dehydrogenase	Glutaric acedemia type 2; ethylmalonic adipic acid in blood and urine	Hypoglycemia; dystonia; dyskinesia; metabolic acidosis; central nervous system degeneration with encephalopathy; respiratory distress; hyperammonemia; birth defects

*CoA indicates coenzyme A.

Several inherited defects in fatty acid oxidation with monitorable traditional biomarkers have been identified in infants presenting with hypoglycemia. These infants have an impaired capacity to use stored fat as fuel during periods of fasting and readily deplete their hepatic glycogen reserves. Despite the development of hypoglycemia, acetyl-CoA production is diminished, and ketone production is impaired. The hypoglycemia occurring in these conditions is typically characterized as hypoketotic (Table 5), although small amounts of ketones may be produced. Hypoglycemia may also occur in other biochemical disorders, including Reye's syndrome (a disease occurring primarily in children due to viral infection), and is sometimes accompanied by hyperammonemia, metabolic acidosis, and elevated transaminases with or without hepatomegaly.

The most common fatty acid oxidation defect is medium-chain acyl-CoA dehydrogenase deficiency (Table 5). In addition to presenting as nonketotic hypoglycemia or a Reye's-like syndrome, it may present as sudden death or an acute life-threatening event. Many reports of infants diagnosed as having medium-chain acyl-CoA dehydrogenase deficiency have described a history of a sibling who died of sudden infant death syndrome. Fat accumulation in the liver or muscle of any infant dying unexpectedly is strongly suggestive of fatty acid oxidation defect. Very long chain fatty acyl-CoA dehydrogenase deficiency is associated with similar clinical findings, although it may be associated with cardiomyopathy. Infants with this and several other fatty acid oxidation defects may present with cardiac arrhythmias or unexplained cardiac arrest.

Less traditional but well-documented biomarkers of fatty acid oxidation defects are also present. The accumulation of fatty acyl-CoA in these patients leads to a secondary carnitine deficiency, probably as a result of excretion of excess acylcarnitines into the urine. Urine organic acid analysis and measurement of carnitine and acylcarnitine in serum/plasma are very helpful in the initial screening for defects in fatty acid oxidation. These studies are sufficient to establish the diagnosis of medium-chain acyl-CoA dehydrogenase deficiency, which is associated with the presence of a characteristic metabolite, octanoylcarnitine, on the acylcarnitine profile. Sometimes enzymatic assays may be necessary for definitive diagnosis of some of the fatty acid oxidation defects.

Jaundice and Liver Dysfunction

Jaundice or other evidence of liver dysfunction has been found in several IMDs in infancy. For most of the IMDs associated with jaundice, the elevated serum bilirubin is of the direct-reacting type. This generalization does not include those IMDs of erythrocyte metabolism, such as glucose-6-phosphate dehydrogenase deficiency or pyruvate kinase deficiency, which are occasionally responsible for hemolytic disease in the newborn. The best-known metabolic disease associated with jaundice is galactosemia, in which deficiency of the enzyme galactose-1-phosphate uridyl

Table 5. Fatty Acid Metabolism Disorders and Organic Acedemias/Acidurias*

Deficiency	Disorder	Clinical Feature
Short-chain acyl-CoA dehydrogenase	Ethylmalonic aciduria; carnitine and acyl carnitines in plasma	Metabolic acidosis; failure to thrive; developmental delay; seizures; myopathy; muscle weakness with wasting
Medium-chain acyl-CoA dehydrogenase	C6-C14 dicarboxylic aciduria; adipic, sebacic, seburic, oxalic acids in urine and serum; carnitine and acyl carnitines (octanoyl carnitine) in plasma	Hyperammonemia; lethargy; nonketonic hypoglycemia and coma; peripheral lobular fatty changes in the liver; unresponsiveness to carnitine therapy
Long-chain acyl-CoA dehydrogenase	C16 and C18 fatty acids in adipose; dicarboxylic acids in urine; carnitine and acyl carnitines	Nonketonic hypoglycemia; gastrointestinal problems due to celiac disease; cardiomegaly; hepatosplenomegaly; cardiorespiratory arrest resulting in death
Very long chain acyl-CoA dehydrogenase	C14:1; C14; carnitine and acyl carnitines in plasma	Hypoketonic hypoglycemia; hepatocellular dysfunction; cardiomyopathy; lipid accumulation; increased urinary levels of adipate and sebacate
Long-chain carnitine palmitoyltransferases (type 1 and 2)	C0 and C16, Low C18; carnitine and acyl carnitines	Hypoketonic hypoglycemia; hepatomegaly; hepatic dysfunction; seizure; coma; cardiomegaly in type 2 disease

*CoA indicates coenzyme A.

transferase (Table 1) results in accumulation of galactose-1-phosphate and other metabolites such as galactitol that are thought to have a direct toxic effect on the liver and other organs. Galactosemia usually causes no symptoms at birth, but jaundice, diarrhea, vomiting, and cataract formation soon develop and the baby fails to gain weight. Hypoglycemia may be observed. The disease may present initially with indirect hyperbilirubinemia resulting from hemolysis secondary to high levels of galactose-1-phosphate in erythrocytes. In addition, the effects of acute galactose toxicity in the brain may sometimes cause the CNS symptoms to predominate. *Escherichia coli* infections are common in untreated galactosemic infants, and death can occur at as early as 1 to 2 weeks of age from severe *E coli* infections. The American Liver Foundation recommends that all infants who develop jaundice be considered for galactosemia.

Another disorder that may be associated with neonatal jaundice is $_1$-antitrypsin deficiency. A determination of serum $_1$-antitrypsin is part of the initial evaluation of children presenting with this syndrome. Hereditary tyrosinemia is also the cause of liver disease in early infancy. The biochemical presentation of this disorder includes marked

Table 6. Inborn Errors of Nucleic Acid (Purine) Metabolism

Deficiency	Clinical Feature
Xanthine oxidase	Hypouricemia; radiolucent stones; acute renal failure; myopathy
Adenine phosphoribosyltransferase	Acute renal failure; crystalluria; recurrent urinary tract infection; hematuria; radiolucent stones
Hypoxanthine-guanine phosphoribosyltransferase	Acute renal failure; crystalluria; hematuria; recurrent urinary tract infection; gout; radiolucent stones; at 3 months+, delayed motor development; at 8 months+, central nervous system involvement; for adults, gout, radiolucent stones, acute renal failure
Purine nucleoside phosphorylase	At 3 months+, delayed motor development; viral infections; lymphopenia; T-cell dysfunction
Adenosine deaminase	Failure to thrive; diarrhea; thrush; lymphopenia; severe combined immunodeficiency
Adenylosuccinase	Severe psychomotor retardation; axial hypotonia; epilepsy; autism
Myoadenylate deaminase	Hypotonia; cardiomyopathy; for adults, exercise intolerance, myopathy

Table 7. Inborn Errors of Nucleic Acid (Pyrimidine) Metabolism*

Deficiency	Disorder	Clinical Feature
Uridine monophosphate synthase	Orotic acid or orotidine in urine	Hypochromic megablastic anemia; crystalluria; retarded development
Uridine monophosphate hydrolase-1	Pyrimidine nucleotides elevated in red blood cells	Nonspherocytic hemolytic anemia with basophilic stippling; hemoglobinuria
Dihydropyrimidine dehydrogenase	Abnormal metabolite 5-hydroxymethyluracil with massive increases in thymine and uracil in urine	Retarded development; epilepsy; microcephaly; for adults, 5-fluorouracil toxicity
Dihydropyrimidinase	Thymine, dihydrothymine, uracil, dihydrouracil in urine	Retarded development; seizures; microcephaly; spastic quadriplegia
CDP choline phosphotransferase	Elevated CDP-choline or CDP-ethanolamine in red blood cells	For infants/adults, hemolytic anemia
Thymidine phosphorylase	Elevated thymidine, uridine, deoxyuridine in plasma or urine; Mt DNA depletion	Mitochondrial neurogastrointestinal encephalomyopathy, ocular and skeletal myopathy with gastrointestinal symptoms
Thymidine kinase 2	Mt DNA depletion	Severe mitochondrial myopathy
Ureidopropionase	Dihydrothymine, dihydrouracil; moderately elevated N-carbamyl-b-alanine, N-carbamyl-b-aminoisobutyric acid in urine, plasma, and cerebrospinal fluid	Hypotonia; developmental delay; optic atrophy

*CDP indicates cytidine-5′-diphosphocholine; Mt, mitochondrial.

elevations of plasma tyrosine and methionine and generalized aminoaciduria with increased excretion of tyrosine. Determination of succinylacetone in the urine is helpful in diagnosing the disorder.

Inherited defects of transporters play a big part in the cholestatic disease process (Table 7).[45,46] For example, mutations in the gene for the bile salt export pump cause progressive familial intrahepatic cholestasis (PFIC). Another example is mutations in transporters causing adult-onset diseases such as intrahepatic cholestasis during pregnancy.[45,46] Neonatal cholestasis is defined as prolonged conjugated hyperbilirubinemia that occurs in the newborn period. It results from diminished bile flow and/or excretion of conjugated bilirubin from the hepatocyte into the duodenum. The primary

Table 8. Urea Cycle Disorders

Deficiency	Disorder	Clinical Feature
N-Acetylglutamate synthase	Hyperammonemia that may be accompanied by high plasma concentrations of alanine and glutamine	Lethargy; persistent vomiting; poor feeding; hyperventilation; enlarged liver; seizures
Carbamoyl phosphate synthetase	Hyperammonemia; citrullinemia; respiratory alkalosis	Lethargy; coma; seizures; vomiting; poor feeding; hyperventilation; hepatomegaly
Ornithine transcarbamylase	Hyperammonemia; respiratory alkalosis; elevated orotic acid in urine	Seizures; vomiting; poor feeding; hyperventilation; hepatomegaly
Arginosuccinate synthetase	Citrullinemia	Lethargy; coma; seizures; vomiting; poor feeding; hepatomegaly
Arginosuccinate lyase	Elevated arginosuccinic acid in urine	Lethargy; seizures; vomiting; poor feeding; hyperventilation; hepatomegaly
Arginase	Markedly elevated plasma arginine, lactate, and CSF glutamine, and modestly elevated blood ammonia	Delayed development; protein intolerance; spasticity; loss of muscle control; seizures; irritability

CSF indicates cerebrospinal fluid.

Table 9. Cytochrome P450 Deficiency and Fatty Acid Metabolism Disorders*

CYP Gene Mutation	Disorder/Clinical Feature
CYP1B1	Primary congenital glaucoma (bupthalmos)
CYP4A, 4B	Defects in salt metabolism; water balance leading to arterial hypertension
CYP5A1, 8A1	Defects leading to clotting and inflammatory disorders, coronary artery disease, and pulmonary hypertension
CYP7A1	Hypercholesterolemia; resistance to statin drugs
CYP7B1	Severe hypercholesterolemia and neonatal liver disease
CYP11A1	Lipoid adrenal hyperplasia, occasional CAH
CYP11B1	Occasional CAH
CYP11B2	Corticosterone methyloxidase deficiency type 1 or 2; occasional CAH
CYP11B1, 11B2	Chimeric enzymes causing glucocorticoid remediable aldosteronism; occasional CAH
CYP17A1	Mineralocorticoid excess syndromes; glucocorticoid and sex hormone deficiencies; association with increased risk of prostate cancer and benign prostatic hypertrophy
CYP19A1	Loss of function: virilization in females, hypervirilization in males, occasional CAH; gain of function: gynecomastia in young males
CYP21A2	More than 90% of all CAH is due to this gene mutation
CYP24A1	Hypervitaminosis D
CYP27A1	Cerebrotendinous xanthomatosis
CYP27B1	Vitamin D–dependent rickets type 1

*CYP, cytochromes P450; CAH, congenital adrenal hyperplasia.

clinical feature is an elevated conjugated bilirubin level >5 mg/dL of the total bilirubin level. Cholestasis must always be considered in newborns with prolonged jaundice lasting more than 14 to 21 days.

Other canalicular transporters include multidrug-resistant proteins MRP2 and MDR3, which transport conjugated bilirubin and phospholipids, respectively, into bile. Deficiencies in these enzymes have been shown to cause Dubin-Johnson syndrome (with conjugated hyperbilirubinemia and jaundice) and PFIC, respectively (Table 7).[45,46]

In contrast to hepatic disorders in which there is an elevation of the direct-reacting bilirubin,[47] a persistent elevation of indirect bilirubin beyond the limits of physiologic jaundice, without evidence of hemolysis, suggests Crigler-Najjar syndrome.[48] The hyperbilirubinemia in this disorder is related to a partial or complete deficiency of glucuronyl transferase, the liver enzyme responsible for the conjugation of insoluble bilirubin to water-soluble bilirubin diglucuronide. Some antiviral drugs such as atazanavir[49] or indinavir[50] have been shown to increase bilirubin, without any hepatocellular toxicity, which is attributed to the inhibition of glucuronosyltransferase 1A1 by these drugs.

Lipid and Lysosomal Storage Disorders

Lipid storage disorders are due to an inherited deficiency of a lysosomal hydrolase that leads to lysosomal accumulation of the enzyme's specific sphingolipid substrate.[31] Disorders include GM_1 gangliosidosis, GM_2 gangliosidosis, Gaucher's disease, Niemann-Pick disease, Fabry's disease, fucosidosis, Schindler's disease, metachromatic leukodystrophy, Krabbe's disease, multiple sulfatase deficiency, Farber disease, and Wolman's disease.[30,31] Many of these diseases do not typically present in early infancy. Among those that occasionally may be associated with hepatosplenomegaly in the first few months of life are GM_1 gangliosidosis, Gaucher's disease, Niemann-Pick disease, and Wolman's disease. Newborns with the typical features of these syndromes, such as coarse facial features, hepatosplenomegaly, skeletal abnormalities, and hernias, are more likely to have GM_1 gangliosidosis or a mucolipidosis, such as I-cell disease. β-Glucuronidase deficiency, classified as mucopolysaccharidosis type VII, presents with an infantile form of sialidosis and is typically associated with fetal hydrops.[31,50] The clinical manifestations of sialidosis may be so severe in utero that fetal hydrops develops.[51] Fetal hydrops is a serious fetal condition defined as abnormal accumulation of fluid in 2 or more fetal compartments, including ascites, pleural effusion, pericardial effusion, and skin edema. Clinical features of β-glucuronidase deficiency may be virtually indistinguishable from those seen in Hurler and Hunter syndromes.[52] The genetic defect involved in faulty degradation[53] of mucopolysaccharides in these syndromes is not known but may involve altered hexosaminidase A activity.[54]

If one of these disorders is suspected, urine screening tests for mucopolysaccharides and oligosaccharides are performed, but false-positive mucopolysaccharide test results

Table 10. Genetic Defects in Transporters and Related Disorders*

Transporter Gene Mutation	Substrate	Disorder/Clinical Feature	Biomarker
PFIC type 1	?	PFIC Type 1 (steatorrhea, diarrhea, jaundice, hepatosplenomegaly, and fatal liver failure within the first 10 years of life)	GGT in serum? Bile salts in serum and bile
BSEP (sPGP)	Bile salts	PFIC type 2 (similar to PFIC type 1, without watery diarrhea)	GGT in serum? Bile salts in serum and bile; phospholipid and cholesterol in bile?
MDR3/MDR2	Phosphatidylcholine	PFIC type 3 (bile duct proliferation and cirrhosis; portal hypertension; hepatosplenomegaly; pruritus)	GGT in serum
MRP2 (cMOAT)	Amphipathic drugs (anionic and neutral)	Dubin-Johnson syndrome (nonhemolytic hyperbilirubinemia; lysosomal accumulation of black pigment)	Bilirubin; urinary coproporphyrin
ABCG5	Phytosterols	Sitosterolemia (tendon xanthomas; accelerated atherosclerosis; hemolytic episodes; arthritis; arthralgia)	Sitosterol in bile and plasma

*PFIC indicates progressive familial intrahepatic cholestasis; GGT, gamma-glutamyltranspeptidase; BSEP, bile salt export pump; sPGP, sister P-glycoprotein; MDR, multi drug resistance protein; cMOAT, multi specific organic anion transporter; ABCG, adenosine 5'-triphosphate-binding cassette-Type G protein.

are commonly observed in neonates. The definitive diagnosis of most lysosomal storage disorders is made by appropriate biochemical studies on leukocytes or cultured skin fibroblasts.[31]

Altered Morphological Features

Thalidomide was the first drug withdrawn from the market in 1960s because of its teratogenic effects.[55] Pregnant mothers who took this drug for morning sickness gave birth to children with several morphological defects, including defects to limbs and arms. However, to date, the exact mechanism(s) by which thalidomide caused teratogenic effects is not clearly understood.[56] Exposure to alcohol, another teratogen, during fetal life may result in several disturbances ranging from growth retardation to behavioral abnormalities later in life. The developing brain is particularly susceptible to prenatal exposure to alcohol. Those affected may present mental and motor retardation, hyperactivity, and poor attention span. It has been proposed that in fetal alcohol syndrome, the reactive acetaldehyde metabolite may inhibit fetal PDH, thus leading to malformations.[31]

Some IMDs may be associated with visible birth defects, suggesting that metabolic derangements in utero may disrupt the normal process of fetal development.[31] These include Zellweger syndrome (a rare congenital disorder characterized by the reduction or absence of peroxisomes in the cells of the liver, kidneys, and brain),[57,58] neonatal adrenoleukodystrophy, and associated variant conditions, all of which are associated with congenital hypotonia and dysmorphic features such as epicanthal folds, Brushfield's spots, large fontanels, simian creases, and renal cysts. Multiple defects in peroxisomal enzymes, including fatty acid oxidation and plasmalogen (glycerol-based phospholipid in which a fatty acid function is replaced by a fatty aldehyde) synthesis, appear to be involved in these birth defects. Patients with glutaric acidemia type II (Table 4) are identified by a characteristic phenotype, including a high forehead, hypertelorism, low-set ears, abdominal wall defects, enlarged kidneys, hypospadias, and rocker bottom feet.[31] An energy-deficient mechanism has been suggested to explain these findings. Several of the organic acidemias, such as mevalonicaciduria and 3-OH-isobutyric aciduria, as well as PDH deficiency, have been associated with multiple dysmorphic features also. Patients with nonketotic hyperglycinemia frequently have agenesis of the corpus callosum and may have gyral malformations related to defects in neuronal migration.[31] Agenesis of the corpus callosum is also seen in PDH deficiency. The dysmorphic findings in PDH deficiency may strongly resemble those observed in fetal alcohol syndrome.

The Smith-Lemli-Opitz syndrome, a genetic defect of 7-dehydrocholesterol reductase deficiency,[59] is associated with

a wide range of malformations, including dysmorphic faces, cleft palate, congenital heart disease, hypospadias, polydactyly, and syndactyly.[31] This disorder is associated with decreased levels of plasma cholesterol and markedly elevated levels of the cholesterol precursor 7-dehydrocholesterol.

Abnormal eye pathology is also associated with many of the IMDs. Cataracts are associated with galactosemia, Zellweger syndrome, Lowe syndrome, and several other conditions.[58] Dislocated lenses are associated with homocystinuria, molybdenum cofactor deficiency, and sulfite oxidase deficiency. Peroxisomal disorders are associated with retinal degenerative changes. Other ocular abnormalities that may be associated with IMDs include corneal clouding and congenital glaucoma.

Interestingly, some of the dysmorphic features discussed here are also observed during teratology studies in animals during drug development. However, a direct association of a drug or its metabolite with an effect on a metabolic pathway, leading to these observations, has yet to be presented in the literature.

Analytical Techniques Used in the Metabonomics and in the Characterization of IMDs

Analytical techniques applied in the characterization of IMDs appear to be more advanced than those applied in the pharmaceutical industry. Several mass spectrometry techniques have been already used in pediatric hospitals in the diagnosis of IMDs. For example, many laboratories have used tandem mass spectrometry for the characterization of amino acid and fatty acid metabolism disorders, including organic acidemias.[32-37] Although there is no one unique technique or method that can be applied to characterize the entire metabonome, the symptoms/clinical features of IMDs help researchers select and apply techniques. In recent years a high-resolution accurate mass spectrometer, the LTQ-Orbitrap (a hybrid tandem instrument combining a linear ion trap mass spectrometer with a new Fourier transform–FT mass analyzer called Orbitrap), has been revolutionizing the use of mass spectrometry for metabonome characterization.[60] Another emerging technology, matrix-assisted laser desorption/ionization, is revolutionizing the use of mass spectrometry for metabonome profiling via direct examination of tissues by imaging.[61] Both techniques have their limitations but will continue to evolve.

Nicholson and colleagues have applied NMR technology for characterizing the metabonome of toxic effects of drugs in animal models.[62-66] NMR is a relatively insensitive technique compared with mass spectrometry. However, high-resolution NMR spectroscopy is an inherently quantitative technique that can report on hundreds of compounds in a single measurement.[67] Although many advances have been made in NMR instrumentation, the technology is still evolving, with the latest cryogenically cooled probes and capillary probes pushing the detection limits to nanomolar levels and allowing for lower sample volumes.[67] Interestingly, the magic angle spinning NMR technique allows intact tissues and cells to be examined for metabonome profiles with little or no preparation and on as little as 20 mg of tissue.[67]

Clearly, both mass spectrometry and NMR techniques can be applied for characterization of the metabonome.[67,68] One major challenge has been in the acquisition and processing of raw analytical data. Characterizing a metabonome of 3000 metabolites is not a trivial matter, and therefore several pattern recognition tools have been employed to differentiate and fingerprint the specific metabolites that may be elevated because of the insult from the toxin.

Another challenge is to develop a model that combines all the information, including pathology, genomic data, and metabonomic data, to generate a meaningful, functionally relevant model for unraveling the target(s) of toxic insult. This will clearly present a challenge in the future. In the meantime, the pharmaceutical industry should focus on applying "knowledge-based" metabonomics, particularly insights gained from IMDs.

Can Metabonomics (Advanced Clinical Chemistry) Replace Traditional Clinical Chemistry?

During preclinical and clinical drug development, the pharmaceutical industry uses only 20 metabolites and 7 enzymes as a routine clinical chemistry panel (Table 11), from the ~3000 metabolites and 100 000 proteins present in animals and humans. In contrast, pediatric hospitals use more advanced clinical chemistry to diagnose an infant presenting with IMDs. One may wonder why the diagnosis and mechanistic understanding of toxicologic pathology has been hampered so severely in the pharmaceutical industry. Is it perhaps because of the limited clinical chemistry parameters used? In addition, with recent advances and rapidly evolving NMR and mass spectrometry technologies, the characterization of the metabonome appears to have become much simpler than ever before.

Regulatory barriers, both real and perceived, slow the development of new or exploratory markers. Arguments that there is no historical database or normal ranges, or no real validation of many of the available ranges, and the fear that review boards will not know how to interpret any "positive" findings, slow both the development of novel biomarkers and possibly the expanded use of markers associated with some of the inborn errors of metabolism described above. Safe harbor, currently afforded to genomic data by the US Food and Drug Administration, should be

Table 11. Routine Panel of Clinical Chemistry Parameters Measured During Preclinical and Clinical Drug Development

Metabolite/Chemical	Enzyme/Protein
Glucose	Alanine-aminotransferase
Urea nitrogen	Aspartate-aminotransferase
Creatinine	Amylase
Uric acid	Alkaline phosphatase
Bilirubin, total	Gamma-glutamyl transpeptidase/transferase
Phosphorus	Creatine kinase and isozymes
Calcium, total	Lactate dehydrogenase and isozymes
Magnesium	Albumin
Iron	Total protein
Sodium	
Cholesterol—total, high-density lipoprotein, and low-density lipoprotein	
Triglycerides	
Homocysteine	

extended to encourage a more aggressive approach to expanding biomarker development.

Conclusions

The use of metabonomics as a tool is quickly evolving in the pharmaceutical industry for rapid discovery of biomarkers and for preclinical and clinical safety and efficacy evaluation. The available metabonomics biomarkers from the IMD database should help toxicologists characterize the mechanisms of drug-induced toxicities. Toxicologists in preclinical development should soon replace routine clinical chemistry with metabonomics as advanced clinical chemistry in order to help them characterize the drug-induced toxicities and aid in selecting clinical biomarkers for safety monitoring during clinical development. In combination with pharmacogenetics, this research will greatly help in minimizing ADRs by monitoring both the genotype and the phenotype during clinical development and postmarketing pharmacovigilance. Application of metabonomics techniques in the diagnosis of preclinical and clinical toxicology is immediately warranted to bridge the gaps between preclinical and clinical development, and clinical development and market.

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